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Three nuclei in pollen grains.

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(WITH PLATE XVI.)

The pollen under consideration was obtained by placing twigs of Sambucus racemosa in a tall bell jar of water on March 3d and keeping them in a warm sunny room. The experiment was begun primarily to illustrate to students the effect of coating the cut surface of the severed twig with asphalt or a similar substance impervious to water. There were four similar twigs, each eighteen inches long, placed in the water. Two of them had their cut surfaces coated with varnish, while the remaining two were set in the liquid with the freshly cut ends fully exposed. The asphalt used was that employed for cementing cover glasses to the slides in making permanent microscopic preparations. The buds upon the stems having free cut surfaces quickly began to enlarge, while those of the twigs that had received the varnish did nothing more than slightly swell, and never produced any leaves. The unvarnished stems sent out their characteristic foliage, and at three of the nodes flower clusters of considerable size were developed. It was from these flowers that the pollen was obtained for the observations herein recorded.

The above facts are given because there may be a difference between the behavior of the pollen thus forced before its time and that which would have been produced normally upon the shrubs several weeks later in the season.

When viewed dry, the pollen is about twice as long as broad (20 μ by 40 μ), and exhibits three dark lines or sutures which run lengthwise of the grain. The appearance of the dry grains is shown at *a* in the plate. All the illustrations were drawn with the camera to the same scale, and are magnified two hundred and eighty times. To the left of *p* are two grains showing the surface of the dry grains. The extine is marked with faint irregular lines, resembling a network of minute cells. This slight obstruction to the view quickly disappears when water is added, and at the same time the grains become nearly spherical and lose all signs of the original tripartite nature. When treated with a mixture of half water and glycerine the nuclei are easily seen. On the right of *p* are three grains; the upper one shows an end view with the three division lines of the grain. Below this are two grains in which the nuclei are evident. There seems to be a nucleus for each third of the grain. After pollen has been in pure glycerine for two days the grains become plump and the contents are nearly colorless, except the nuclei, which are large and well defined. Three such grains are shown at *c*. In many instances the nuclei seemed united by their ends, while in others all three were evident.

For germination fresh pollen was placed in a ten per cent. solution of cane sugar in one of a nest of porcelain macerating dishes, and forty hours after the tubes were of the lengths indicated in the plate. The earlier stages of germination are shown at *d*. Usually there was a single tube, but occasionally the tube branched as it issued from the grain. In some cases there were two points of departure, as shown near *a* and at *h*. The course of the tube was rarely in a straight line, but instead became much twisted and contorted during the early part of its growth. Afterwards it expanded into a swollen tip as at *e*, where there is a strong tendency to fork at its enlarged extremity. To the left of *e* is a tube which makes a sharp twist near the middle and afterwards swells into an Indian-club-like tip.

The coloring substances which proved the best for the study of the nuclei were eosin and azo-rubin. The latter is by far the best of all substances used for the demonstration of the nuclei. Nearly all of the tubes showing three nuclei, as given in the plate, were treated with azo-rubin. No nuclei are shown in some of the tubes, and these, as at *e* and *t*, were drawn from eosin-prepared specimens. The aceto-methyl green recommended by Dr. Strasburger proved of very little service in comparison with the azo-rubin. This latter substance almost instantly developed a deep blood-red color in the nucleus, while the remaining portion of the protoplasm was left of a lighter hue.

Observations were repeated from day to day with fresh specimens dipped from the culture dish, and the presence of the three nuclei was demonstrated in hundreds of cases. In fact, in nearly all long tubes the three blood-red bodies would appear upon the application of the coloring reagent. In many instances the three nearly equal-sized bodies were found after all else in the tube had disappeared.

The attention of teachers who give laboratory instruction is called to the exceedingly easy way in which an unlimited supply of material was provided for this study. A thoroughly cleaned porcelain dish three inches in diameter and a quarter of an inch deep received an ounce of ten per cent. sugar solution, and into this a large quantity of the pollen was dusted from the flowers. A second dish was placed over the first so as to prevent evaporation and the ingress of mold germs. The nourishing solution was kept in a warm room, for it was yet cold out of doors and snow covered the ground. New pollen was dusted into the syrup from day to day so that all stages of the tubes might be obtained at any time. A preparation for the microscope was made by simply dipping out a drop of the syrup on the cleaned tip of a scalpel and placing it upon a glass slide. Fully a week elapsed before molds began to interfere with the culture. Even after the fungus filaments were producing an entangled mass the pollen and their tubes could be easily determined by the addition of the azo-rubin. This substance did not affect the mold unless very strong, while it almost instantly colored all the protoplasm of the pollen grains and of their tubes.

No mention is found of pollen grains having three nuclei, so far as the literature of the subject has been reviewed by the writer.[1] Dr. Goodale[2] speaks of only two nuclei, one of which is usually larger than the other. Professor Coulter in his paper[3] shows only two. In like manner Professor Barnes[4] demonstrates but two nuclei. If we follow Strasburger, in his later works, the larger is called the vegetative nucleus. With the case in hand the nuclei are all of nearly equal size. As a rule they pass out into the tube early in its development, and generally may be found in the lower half, if not within the club-shaped extremity. When only two nuclei were found in a grain or its tube, one was frequently larger than the other. This fact has led to the suggestion that the larger or vegetative nucleus may undergo a process of division early in the development of the pollen grain. In some of the immature grains only two nuclei were observed, and these seemed to be united by their slender tips, as indicated above *(c)* in the plate.

In closing, the attention of teachers of physiological bot-

[[1] Strasburger describes and figures pollen grains with three nuclei in his *Neue Untersuchungen*.—EDS.]

[2] Goodale's Physiological Botany, p. 428.

[3] Pollen spores of Tradescantia Virginica. BOT. GAZ. XI, p. 10.

[4] Process of Fertilization in Campanula Americana. BOT. GAZ. X, p. 349.

any is again called to the very easy manner in which a large supply of germinated pollen was provided for the study here outlined. The same method has proved successful in several other cultures.

Fertilization of Calopogon parviflorus Lindl.[1]

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While at Orlando, Florida, in February, 1887, I found this plant very common in pine barrens.

The flower is interesting from the fact that the ovary is not twisted, so that the labellum occupies the upper side and the column the lower. These parts are at right angles to each other, the labellum being erect and the column curving outward and a little downward. The labellum gradually narrows to a winged basal portion, on which it is hinged. In the wind it often bends forward: in old flowers it falls from its own weight and lies upon the column. In front it is furnished with a conspicuous crest of club-shaped hairs. From above the hairs increase in length so that their tips rise to about the same level. The lower project strongly from the labellum, and many of them are fused together, especially below. When the labellum is bent down upon the column the crest does not reach as far as the stigma. The stigma is at the summit of the column, and is covered with viscid matter. In a little pocket just under the stigma lies the anther, which is two-celled, each cell containing two pollen masses whose grains are lightly connected by threads.

Small bees, Andrenidæ, approaching the flower in front, light upon the crest, when the labellum bends suddenly, so that the dorsal surface of the insect comes down upon the column. The broad, slightly upturned wings of the column keep the body from passing to either side, and so require it to slip off the end. In doing this the body strikes the stigma and is smeared with viscid matter. The pressure of the insect upon the stigma starts the anther from the pocket, so that the ends of the pollen masses are exposed. As the body slips off the end of the column the exposed ends of the pollinia strike the part which is smeared with viscid matter from the stigma, and the pollinia are drawn out and cemented to

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[1] Kindly determined by Dr. Sereno Watson. For an interesting account of the morphology of Calopogon and a figure of C. pulchellus, see "Goodale & Sprague," "Wild Flowers," pp. 73 and 85, and pl. xiv.